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Title: THE LETHAL AND SUBLETHAL EFFECTS OF A ZIRCONIUM PROCESS EFFLUENT ON JUVENILE SALMONIDS

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Acute toxicity bioassays and growth studies, with juvenile salmon as test animals, were used to identify and characterize the major toxic components of a zirconium process effluent (ZPE) produced by Teledyne Wah Chang Albany Corporation.

The major toxic component of the ZPE is ammonia. Although other components of the ZPE are toxic, the major portion of the toxicity can be accounted for by the ammonia concentration, so long as ammonia concentrations remain high. The ZPE was more toxic than ammonium chloride solutions having the same ammonia concentration on the basis of acute toxicity and growth studies.

The growth rate of juvenile chinook salmon (Oncorhynchus tshawytscha) at high consumption rates was higher in ammonium chloride solutions having ammonia concentrations less than 3 mg/liter,

as compared to control groups. Ammonia concentrations greater than 3 mg/liter led to decreased growth rates of juvenile chinook salmon. ZPE solutions caused a decrease in the growth rate of juvenile chinook salmon at ammonia concentrations of 0.3, 1.78, 4.5 and 7.44 mg/liter. The zirconium process effluent reduced the growth rate of juvenile salmonids at concentrations of effluent near to those found in the Willamette River.

The Lethal and Sublethal Effects of a Zirconium Process Effluent on Juvenile Salmonids

by

Everett F. Wilson

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THE LETHAL AND SUBLETHAL EFFECTS OF A ZIRCONIUM PROCESS EFFLUENT ON JUVENILE SALMONIDS

INTRODUCTION

The purpose of this study was to examine lethal and sublethal effects of a zirconium process effluent (ZPE) and its major components on juvenile salmonids. The effluent is a mixture of many substances, of which ammonia is present in concentrations normally ranging from 200 to 600 mg/liter and appears to be the major toxic component.

Ammonia has long been recognized as a common constituent of many industrial and sewage wastes (Clark and Adams, 1913). Its acute toxicity to aquatic organisms has been extensively studied. Wuhrmann et al. (1947), Wuhrmann and Woker (1948), and Downing and Merkens (1955) have demonstrated that it is unionized ammonia, not the ionized form, that is responsible for the toxicity of ammonia solutions.

The effects of sublethal concentrations of ammonia on aquatic organisms have not been extensively studied. The effect of ammonia on the growth of fish has been examined in relation to the accumulation of metabolic products in hatchery ponds (Phillips et al., 1949, 1950; Burrows, 1964). The growth rates of juvenile brook trout (Salvelinus fontinalis) and juvenile chinook salmon (Oncorhynchus

tshawytscha) were lower in ponds having high ammonia concentrations. Rainbow trout (Salmo gairdneri) exhibited a diuretic response when exposed to ammonia concentrations greater than 12 percent of the threshold LC_{50} (Lloyd and Orr, 1969). Flis (1968) found that exposure of carp (Cyprinus carpio) to 0.11 mg/liter unionized ammonia for 35 days caused severe liver and kidney damage, which appeared to be associated with disruption of blood vessels. Burrows (1964) found damaged gills in juvenile chinook salmon exposed to low ammonia and urea concentrations in hatchery ponds. Subsequent recovery of the gill epithelium occurred in clean water at 14 C but not at 6 C. Reported studies do not clearly establish the effect on the growth of fish of ammonia alone or ammonia in combination with other constituents of effluents.

In this study, the acute toxicity of the zirconium process effluent (ZPE) over a range of pH values was first determined. The hypothesis that ammonia was the major cause of acute toxicity was evaluated by two approaches. The acute toxicity of ammonium chloride solutions and solutions of ZPE having identical ammonia concentrations were compared at four pH levels; and acute toxicity bioassays with ZPE having different ammonia concentrations were conducted monthly, multiple linear regression analysis being used to compare toxicity (dependent variable) and the chemical constituents of the waste (independent variable).

Fish in receiving water may be exposed to concentrations of either ZPE or ammonia that are not acutely toxic. Growth rates of juvenile salmon were employed as a measure of the sublethal toxicity of ZPE and ammonia.

METHODS AND MATERIALS

Chinook salmon (O. tshawytscha) and coho salmon (O. kisutch) were the test animals used in the experiments. The coho salmon used in experiments A-1 through A-14 were collected either from the Oregon Fish Commission hatchery at Fall Creek or from Tobe Creek, a tributary of the South Fork of the Alsea River. The spring chinook salmon used in experiments B-1 through B-27 and C-1 through C-3 were reared from eggs collected at the Oregon Fish Commission hatchery on the South Santiam River.

The dilution water used in the experiments came from a small spring fed stream. Hardness of this water ranged from 50 to 115 mg/liter as $CaCO_3$; alkalinity ranged from 80 to 120 mg/liter as $CaCO_3$. The unadjusted pH of the dilution water was about 7.0 in winter and near 7.8 in the summer. Water temperature, before control, ranged from 18 C in summer to 5 C in winter.

The zirconium process effluent was collected the day before a set of bioassays was to be initiated. The ZPE for use in the growth studies was stored in two 500-gallon fiberglass tanks and cooled to 2 C $\pm$ 1. The ZPE is treated with lime for ammonia removal and then flows through a clarifier and two settling basins, before entering a small stream that flows into the Willamette River. The ZPE for the experiments was collected from the outfall of the second settling

basin. Ammonia concentrations were determined by the Macro-Kjeldahl method (Environmental Protection Agency, 1971). Other constituents of the effluent were determined by Teledyne Wah Chang analytical chemists.

In this paper, Kjeldahl ammonia ($NH_3+NH_4^+$) will be called ammonia. The unionized or toxic portion of the ammonia solution will be referred to as unionized ammonia.

The dilution apparatus (Chadwick et al., 1972) for the acute toxicity and growth experiments was designed to mix and deliver the desired proportions of water and toxicant to the test chambers. The acute toxicity test chambers were 10 gallon opaque plastic buckets modified to provide flowing water aquaria of 10 liter capacity. The growth chambers were 10-liter plexiglass aquaria arranged in five groups of four each. The exterior surface of the growth aquaria were painted black.

The pH of the test solutions was controlled in two different ways. In experiments A-1 through A-14, pH was controlled by dripping either acid (0.1 N H_2SO_4) or base (0.1 N NaOH) into the dilution water from a small mariotte bottle. In experiments B-1 through B-27 and C-1 through C-3, the pH was maintained at 7.5 by addition of CO_2 followed by vigorous areation. The water temperature was held at 15 C $\pm$ 1 by use of cooling and heating units. Illumination was provided by fluorescent lights, photoperiod being controlled by a timer set

twice each week to coincide with local day length.

Fish to be used in experiments were brought into the laboratory at least two weeks in advance of each experiment. They were kept in 50 gallon aquaria in water of the quality and temperature to be used in the experiment.

The fish to be used in the bioassays were not fed for two days preceding an experiment. One hundred and twenty fish of uniform size were selected for each set of bioassays. One hundred of these fish were randomly placed in the ten test chambers, the remaining 20 being blotted dry, weighed, and measured as a subsample of the test fish.

The number of fish surviving in each chamber was recorded every 24 hours, and dead fish were removed. The 96 hr. median tolerance limit (TL_{50}) was calculated using the graphical method outlined by Doudoroff et al. (1951).

Initial data from the comparative bioassays indicated that the acute toxicity of the effluent and the ammonium chloride solutions was largely expressed in 24 hours, few or no deaths occurring after this period. This agrees with the results of Lloyd (1961), who found that ammonia toxicity was expressed within 250 minutes, for rainbow trout (Salmo gairdneri). On the basis of this, the monitoring bioassays B-1, -2, -3, -4, -7, -8, -9, -10, -15, -16, -19, -20, -25, and -26 were 24 hours in length, the remaining acute toxicity bioassays

being 96 hours in length.

In growth experiments C-1 through C-3, 15 fish nearly uniform in size were placed in each of 20 aquaria. The fish were acclimated for seven days to the aquarium conditions, the experimental temperature, and to the feeding methods. Twenty-one groups of 10 fish each in experiment C-1 and 21 groups of 8 fish each in experiments C-2 and C-3 were selected from the acclimated animals for each experiment. One group was weighed, measured, and dried at 70 C for four days as a subsample of the fish used in each experiment. The subsample was reweighed after drying to estimate the initial dry weight of fish used. The remaining 20 groups were weighed and distributed amongst the 20 experimental aquaria (Table 1).

Flow rates to each chamber were set at 100 milliliters per minute. Two concentrations of ZPE and two concentrations of ammonium chloride, along with a control, were tested simultaneously (Table 1). Four different rations of tubifex worms were fed daily to the four different test groups at each concentration. These were ad libitum, 80, 50, and 20 percent of ad libitum. The ad libitum ration was always large enough to ensure that there would be a small quantity of tubificids left after 24 hours.

A subsample of the tubificids was taken daily and dried at 70 C for four days to provide an estimate of the percent dry weight of tubificids fed each day. The rations were fed for 14 days in

Table 1. Experimental conditions of temperature, pH, ammonia concentration, and size of juvenile chinook salmon.

Experiment	Date	Experiment length days	Temperature	Treatment mg/liter ammonia	Average pH	Mean weight of fish (gms)	Mean fork length of fish (cm)
				NH_4Cl			
C-1	5/17/72	14	15 C	0.30 ± .07	7.52 ± .01	1.43 ± .44	25 ± 1
				4.52 ± .10	7.54 ± .05	1.41 ± .46	24 ± 3
				ZPE			
				0.32 ± .05	7.51 ± .01	1.46 ± .43	23 ± 1
				4.60 ± .10	7.50 ± .03	1.47 ± .40	23 ± 1
				Control	7.51 ± .02	1.44 ± .44	23 ± 1
C-2	6/20/72	12	15 C	NH_4Cl			
				1.00 ± .2	7.50 ± .01	2.86 ± .41	52 ± 3
				2.00 ± .1	7.53 ± .04	2.77 ± .43	51 ± 2
				ZPE			
				1.04 ± .1	7.48 ± .02	2.83 ± .46	50 ± 4
				1.96 ± .2	7.51 ± .02	2.89 ± .46	53 ± 2
				Control	7.52 ± .01	2.76 ± .51	52 ± 6
C-3	8/15/72	12	15 C	NH_4Cl			
				1.90 ± .04	7.58 ± .07	2.75 ± .62	55 ± 6
				7.48 ± .20	7.50 ± .06	2.88 ± .59	59 ± 5
				ZPE			
				1.78 ± .01	7.50 ± .01	2.61 ± .65	52 ± 9
				7.49 ± .40	7.51 ± .02	2.74 ± .61	51 ± 9
				Control	7.50 ± .04	2.76 ± .51	49 ± 6

experiment C-1 and 12 days in experiments C-2 and C-3. The fish were then starved for one day, removed from the aquaria, weighed, dried at 70 C for four days, and weighed again. Fish growth rates were computed by dividing total change in milligrams dry weight, by the product of the mean dry weight in grams and time in days. Food consumption was computed by dividing total dry weight in milligrams of food consumed by the product of mean dry weight of fish in grams and time in days (Warren, 1971).

RESULTS AND INTERPRETATION

Acute toxicity bioassays were used to aid in identification of the major toxic components of the ZPE, to monitor the toxicity of the ZPE over a period of one year, and to characterize the ZPE for growth studies. Monitoring bioassays were performed twice monthly, at a pH of 7.5, from January 1972 through January 1973 (Table 2). The 96 hour TL_{50} for the ZPE ranged from 3.3 to 36.0 percent by volume and appeared to fluctuate with the concentration of ammonia in the ZPE (Figure 1).

A multiple linear regression analysis was used with the bioassay data in an attempt to determine the contribution of each chemical constituent of the effluent to the TL_{50}. In the first models, the analysis used selected the independent variable best explaining variation in the dependent variable, on the basis of the highest R^2 value. Ammonia was found to be the best independent variable ($R^2 = 0.75$), with the regression being significant (F test $p < .01$). Addition of the next best independent variable to the original model did not result in significant improvement (Partial F test $p > .05$). Interaction of two or more constituents was thought to have an effect on the TL_{50}. Therefore new variables were formed both by addition and multiplication of the original variables. The original model with ammonia as the independent variable was still the best model.

Table 2. Median tolerance limits (TL_{50} percent volume) of the ZPE and concentrations of the chemical constituents of the effluent. Experiments conducted at pH 7.5 and 15 C with juvenile chinook salmon.

Date	Experiment number	Median tolerance limit TL_{50} (percent volume) 24 hr	96 hr	Zirconium process effluent constituents Mg/liter NH_3	SCN	F	SO_4	Cl	Na	Ca	MIBK[1]
1-29-72	B-1	3.3	3.3	490	005	08	860	1270	235	0255	095
1-29-72	B-2	3.3	3.3	490	005	08	860	1270	235	0255	095
2-22-72	B-3	10.5	10.5	387	050	31	900	0700	530	0050	090
2-22-72	B-4	10.5	10.5	387	050	31	900	0700	530	0050	090
3-28-72	B-5	5.2		493	040	10	730	0240	220	0110	085
3-28-72	B-6	5.2		493	040	10	730	0240	220	0110	085
4-28-72	B-7	7.5	7.5	414	100	05	780	0700	120	0880	080
4-28-72	B-8	7.5	7.5	414	100	05	780	0700	120	0880	080
5-31-72	B-9	36.0	36.0	023	040	16	630	0440	280	0150	028
5-31-72	B-10	36.0	36.0	023	040	16	630	0440	280	0150	028
6-28-72	B-11	12.5		233	020	10	460	1130	150	0460	020
6-28-72	B-12	13.5		233	020	10	460	1130	150	0460	020
7-28-72	B-13	3.6		593	030	05	520	2200	165	0380	060
8-31-72	B-14	6.1		205	100	06	630	2340	300	1020	053
8-31-72	B-15	3.8	3.8	205	100	06	630	2340	300	1020	053
9-30-72	B-16	6.8	6.5	352	010	10	350	1350	400	0400	070
9-30-72	B-17	7.0		352	010	10	350	1350	400	0400	070
10-9-72	B-18	5.6		325	060	09	480	2020	320	0820	054
10-9-72	B-19	5.6		325	060	09	480	2020	320	0820	054
10-31-72	B-20	5.4	5.4	261	080	08	430	2640	320	1210	080
10-31-72	B-21	5.7	5.7	261	080	08	430	2640	320	1210	080
11-20-72	B-22	11.1		251	080	06	510	2330	360	1200	045
11-20-72	B-23	10.0		251	080	06	510	2330	360	1200	045
11-27-72	B-24	10.0		314	090	04	710	1960	440	1400	090
11-27-72	B-25	10.0	10.0	314	090	04	710	1960	440	1400	090
1-29-73	B-26	6.0	6.0	220	055	06	500	2350	240	1020	045

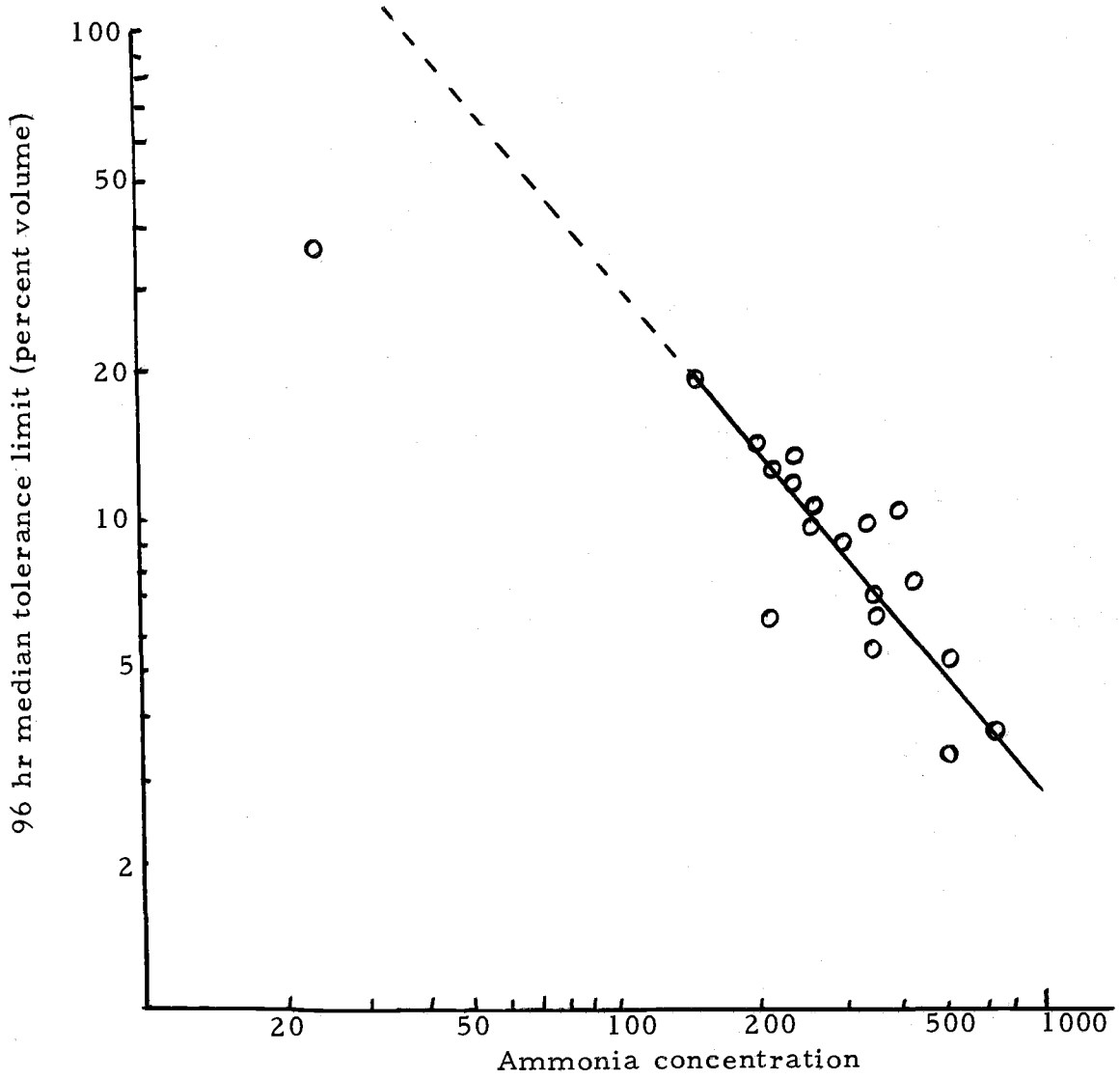

Figure 1. Relationship between the 96 hr TL_{50} (percent volume) and the ammonia concentration of the ZPE used in the monitoring bioassays. Bioassays conducted at a pH of 7.5 and 15 C with juvenile chinook salmon.

Based upon the results of the multiple linear regression analysis, ammonia appears to be the major constituent contributing to the acute toxicity of the ZPE. Nevertheless, the variance of the data about the regression line (Figure 1) suggests that unidentified factors do influence the acute toxicity of this effluent.

Acute toxicity bioassays were used to compare the toxicity of ZPE and ammonium chloride solutions at four different pH levels. The concentration of ammonia yielding the 96 hr TL_{50} decreased as the pH increased (Figure 2). When 96 hr TL_{50}'s were expressed as ammonia concentrations, zirconium process effluent was more toxic than ammonium chloride at each pH level tested. The curves approach each other as the pH increases, and both solutions exhibited nearly the same toxicity at high pH values.

The acute toxicity of the ammonium chloride and ZPE solutions increased approximately nine-fold over the pH range from 7.0 to 8.5. As the pH increases from 7.0 to 8.5, the percentage of the ammonia found in the biologically available unionized form increases approximately 30-fold. Thus, as seen in Figure 3, the toxicity of unionized ammonia appears to decrease as the pH increases. If unionized ammonia is the biologically available portion of an ammonia solution, the concentration of unionized ammonia yielding the 96 hr TL_{50} should be constant over a wide range of pH values.

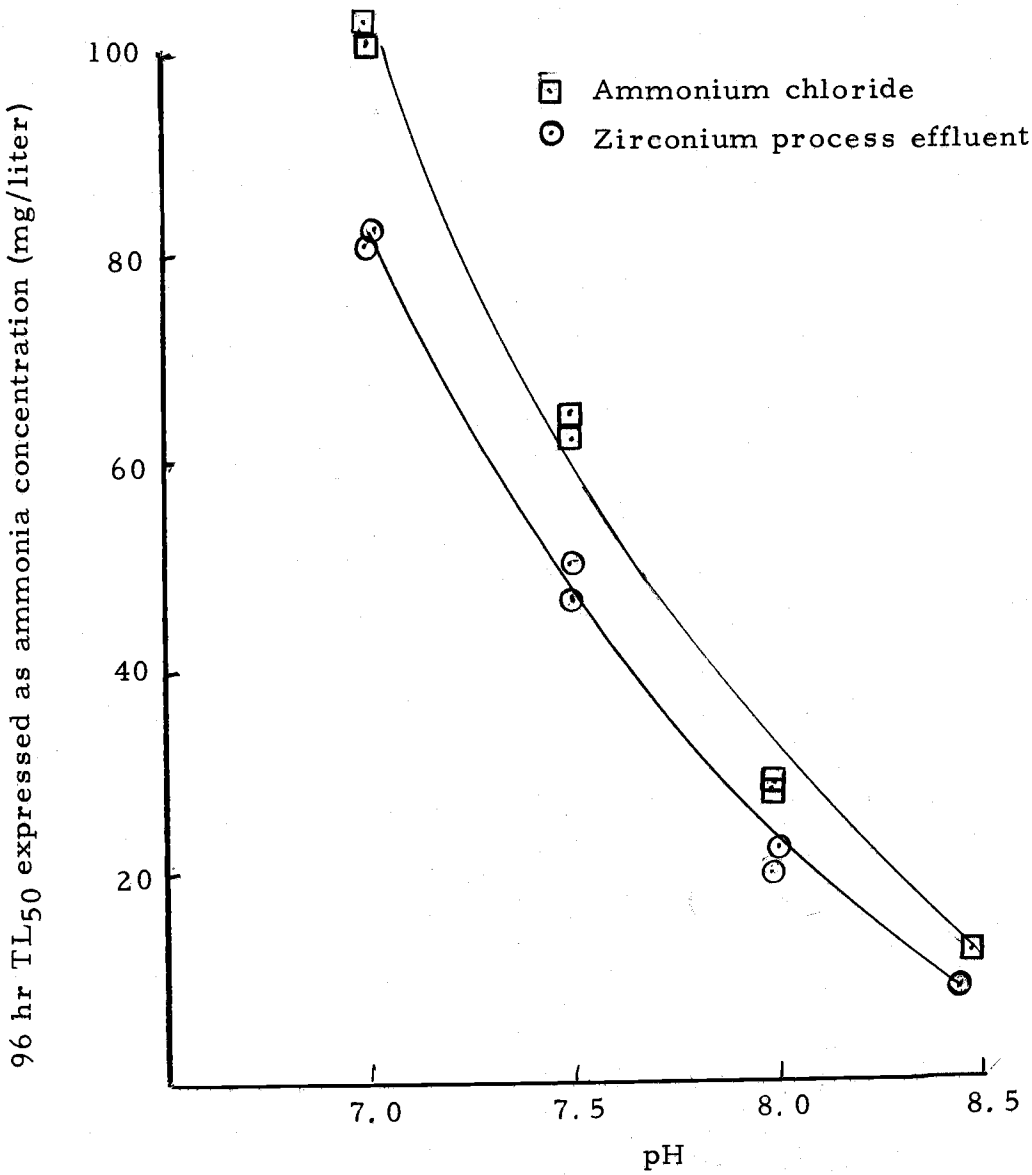

Figure 2. Relationships between the 96 hr TL_{50} ammonia concentration and pH for ammonium chloride and ZPE solutions. Bioassays conducted at 15 C with juvenile coho salmon.

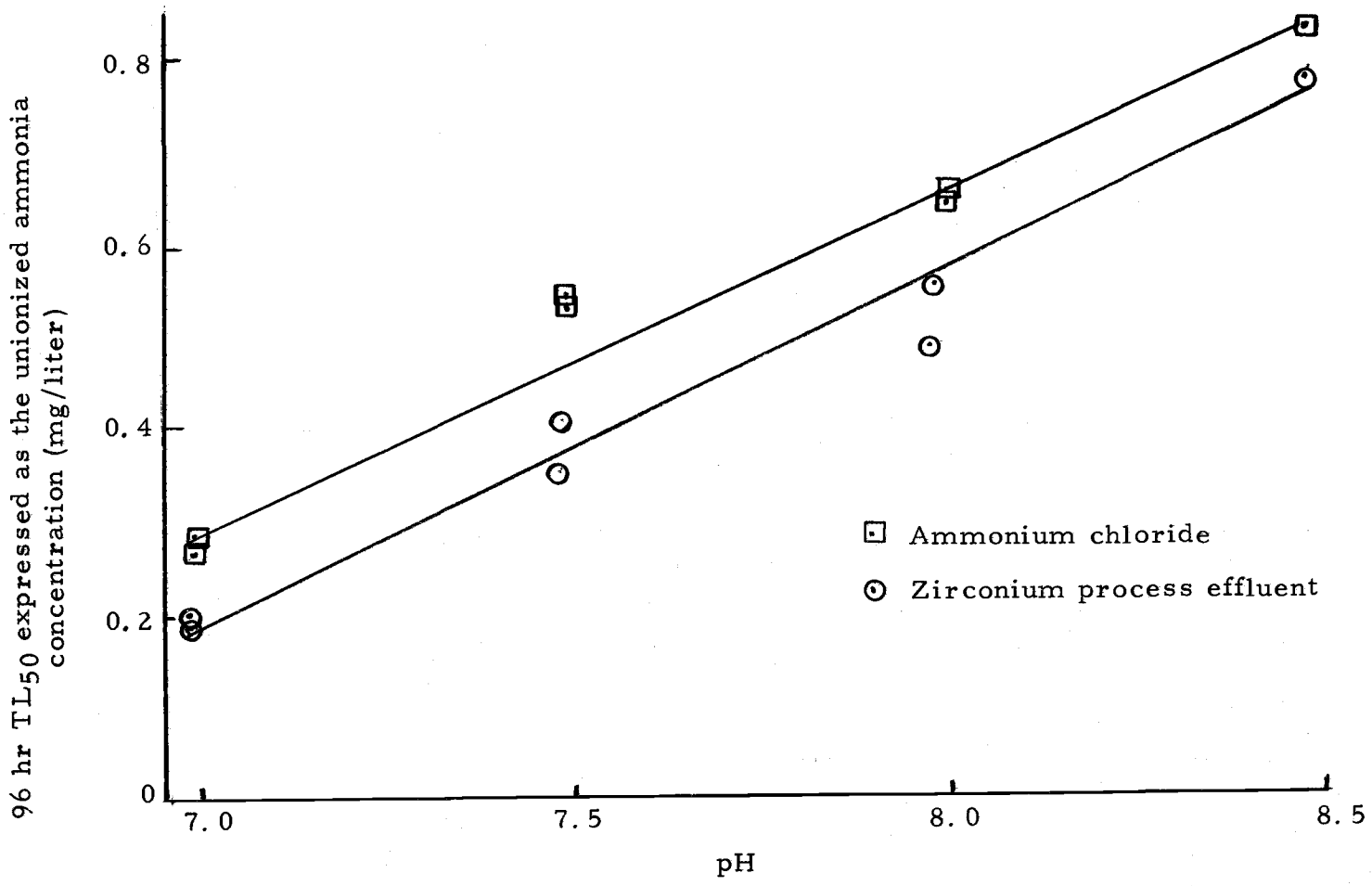

Figure 3. The relationship between the calculated 96 hr TL_{50} unionized ammonia concentration and pH in ammonium chloride and zirconium process effluent solutions. Bioassays conducted at 15 C with juvenile coho salmon.

Lloyd and Herbert (1960) found that CO_2 excreted at the gill surface, when the free CO_2 content of the water was low, lowered the pH of the water in the gill cavity of the fish. The lowering of the pH at the gill surface would shift the ammonia equilibrium towards the ionized form of ammonia. Thus, the concentration of unionized ammonia to which the fish were exposed at the gill surface was in all probability less than the concentration that was calculated from the pH of the solution. Since most ammonia is believed to be absorbed through the gills, the concentration of unionized ammonia at the gill surface is very important.

The concentration of CO_2 present at an instant in time at the distal end of the gill lamellae can be calculated using the formula

$$CO_2 = \frac{\text{Mol. wt. } CO_2}{\text{Mol. wt. } O_2} \times \frac{P}{100} \times C \times R.Q.$$

C = Concentration of dissolved oxygen in the solution

P = The percentage removal of dissolved oxygen from the water by the fish = 80 (Van Dam, 1913).

R.Q. = Respiratory quotient = 0.70 for the diet provided.

According to this formula, the CO_2 concentration at the distal end of the gill lamellae would be 77 percent of the dissolved oxygen concentration in the solution.

Since CO_2 is released along the length of the gill lamellae, a CO_2 gradient is established, the CO_2 concentration being near zero

at the proximal end of the gill lamellae and the calculated concentration at the distal end. The pH of the water in the gill cavity also follows the CO_2 gradient in the gill cavity, the pH being lowest at the distal end of the gill lamellae where the CO_2 concentration is highest. Therefore, to calculate the effect of the CO_2 on the pH in the gill cavity, the average CO_2 concentration was used.

Thus with the average CO_2 concentration, the alkalinity (115 mg/liter as $CaCO_3$), the temperature (15 C), and the recorded pH of the solutions, the average pH at the gill surface can be calculated (Dye, 1952). The TL_{50} as unionized ammonia was recalculated using the adjusted pH values. The variation about the mean is much less when the adjusted pH values are used to compute the TL_{50} than the variance when the measured pH values are used (Table 3).

The bioassays employed for monitoring the acute toxicity of the ZPE and the acute toxicity bioassays used for comparing the toxicity of ammonium chloride and ZPE indicate that ammonia is the major acutely toxic component of the ZPE. The ZPE solutions were more toxic than the ammonium chloride solutions at the same ammonia concentrations and pH's. The additional toxicity exhibited by the ZPE cannot now be attributed to any one of the constituents of the effluent.

Concentrations of ZPE that are acutely toxic to fish do not generally occur in the Willamette River. Growth of fish was used as a measure of the sublethal toxicity of the effluent and of ammonium

Table 3. Relationship between the observed TL_{50} as unionized ammonia and the TL_{50} computed when the effect of CO_2 on pH is taken into account.

Solution pH	TL_{50} unionized ammonia concentration Mg/liter	Average CO_2 concentration Mg/liter	Adjusted pH	Adjusted TL_{50} unionized ammonia concentration Mg/liter
7.0	.2718	3.85	7.00	.2718
7.0	.2800	3.85	7.00	.2800
7.5	.5504	3.85	7.34	.3817
7.5	.5284	3.85	7.34	.3665
8.0	.7115	3.85	7.63	.3082
8.0	.7005	3.85	7.63	.3033
8.5	.8797	3.85	7.86	.2157
	Avg. .5603 ± .2263			Avg. .3039 ± .0568

chloride solutions. In experiment C-1, different groups of juvenile chinook salmon were exposed to 0.3 and 4.5 mg/liter ammonia in ammonium chloride solutions or in ZPE solutions (Figure 4). Fish exposed to 0.3 mg/liter ammonia in ammonium chloride solutions grew more rapidly than the control fish at consumption rates higher than 90 mg/g/day and at rates similar to those of the controls at lower consumption rates (Figure 4a). Fish exposed to 4.5 mg/liter ammonia in ammonium chloride solutions grew more slowly than the controls at consumption rates higher than 115 mg/g/day and at rates similar to those of the controls at lower consumption rates (Figure 4b).

Fish exposed to 0.3 mg/liter ammonia in ZPE solutions grew more slowly than the control fish at consumption rates greater than 100 mg/g/day; at lower consumption rates the exposed fish grew faster than the controls (Figure 4a). Fish exposed to 4.5 mg/liter ammonia in ZPE solutions grew at slower rates than the controls at all consumption rates higher than 80 mg/g/day and faster than the controls at lower consumption rates (Figure 4b).

The growth rate of fish exposed to ZPE solutions was slower than those exposed to ammonium chloride solutions at all except the lowest consumption rates. The difference between the growth of fish exposed to ammonia in ZPE solutions and to ammonium chloride solutions can only be attributed to other toxicants in the effluent.

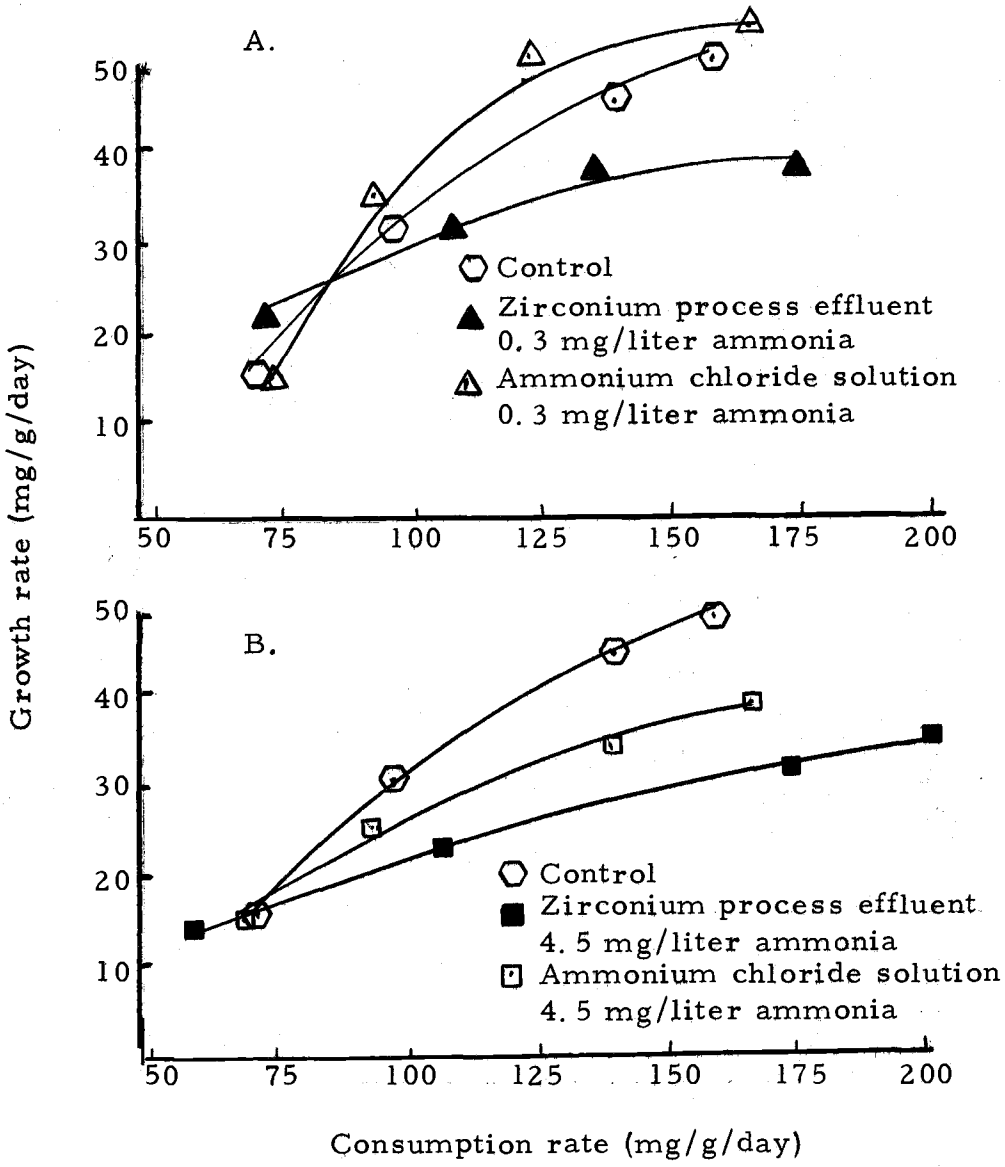

Figure 4. Relationships between growth rate and consumption rate of juvenile chinook salmon exposed to two concentrations of ammonia in either ammonium chloride or ZPE solutions. Experiment conducted at pH 7.5 and 15 C for a period of 14 days.

In experiment C-2, juvenile chinook salmon were exposed to 1 and 2 mg/liter ammonia in ammonium chloride solutions or in ZPE solutions (Figure 5). Curves were drawn only through the control points, points for 1 mg/liter ammonia in ammonium chloride solutions, and points for 2 mg/liter ammonia in ZPE solutions. Smooth curves could not be fitted to the results obtained at the other concentrations (Figure 5b).

The growth rates of fish exposed to 1 and 2 mg/liter ammonia in ammonium chloride solutions were generally greater than those of fish exposed to 1 and 2 mg/liter ammonia in ZPE solutions (Figure 5b). The difference in growth rate can again only be attributed to the other toxicants present in the zirconium process effluent.

In experiment C-3 juvenile chinook salmon were exposed to 1.86 and 7.44 mg/liter ammonia in either ammonium chloride or ZPE solutions (Figure 6). Fish exposed to 1.86 mg/liter ammonia in ammonium chloride solutions grew more rapidly than the controls at all consumption rates (Figure 6a). At 7.44 mg/liter ammonia in ammonium chloride solutions the fish grew more slowly than the control fish at all consumption rates studied (Figure 6b).

Fish exposed to 1.86 mg/liter ammonia in ZPE solutions grew more slowly than the control fish at all consumption rates greater than 50 mg/g/day (Figure 6a). Fish exposed to 7.44 mg/liter ammonia in ZPE solutions grew slower than control fish at all

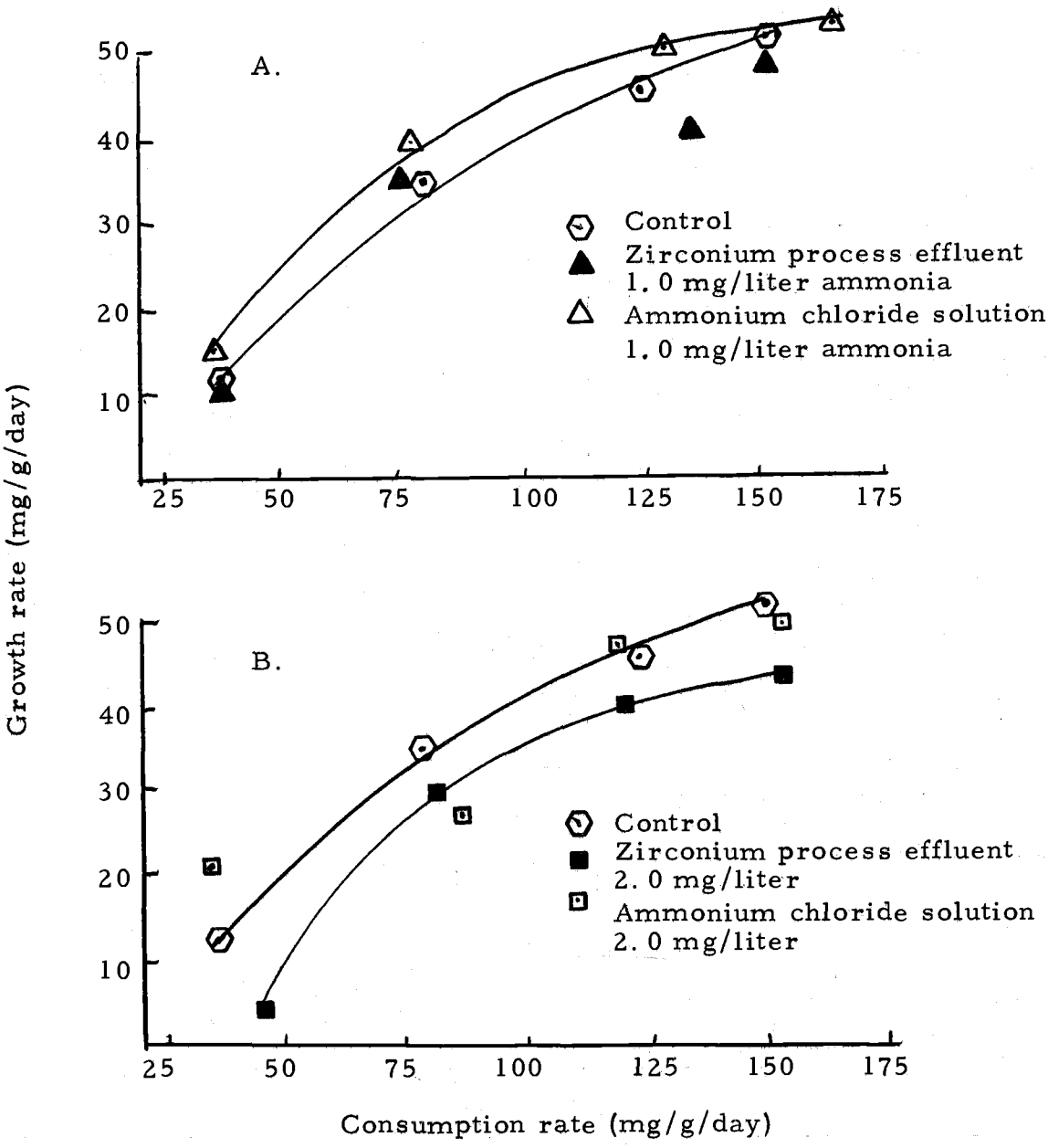

Figure 5. Relationships between growth rate and consumption rate of juvenile chinook salmon exposed to two concentrations of ammonia in either ammonium chloride or ZPE solutions. Experiments conducted at pH 7.5 and 15 C for a period of 12 days.

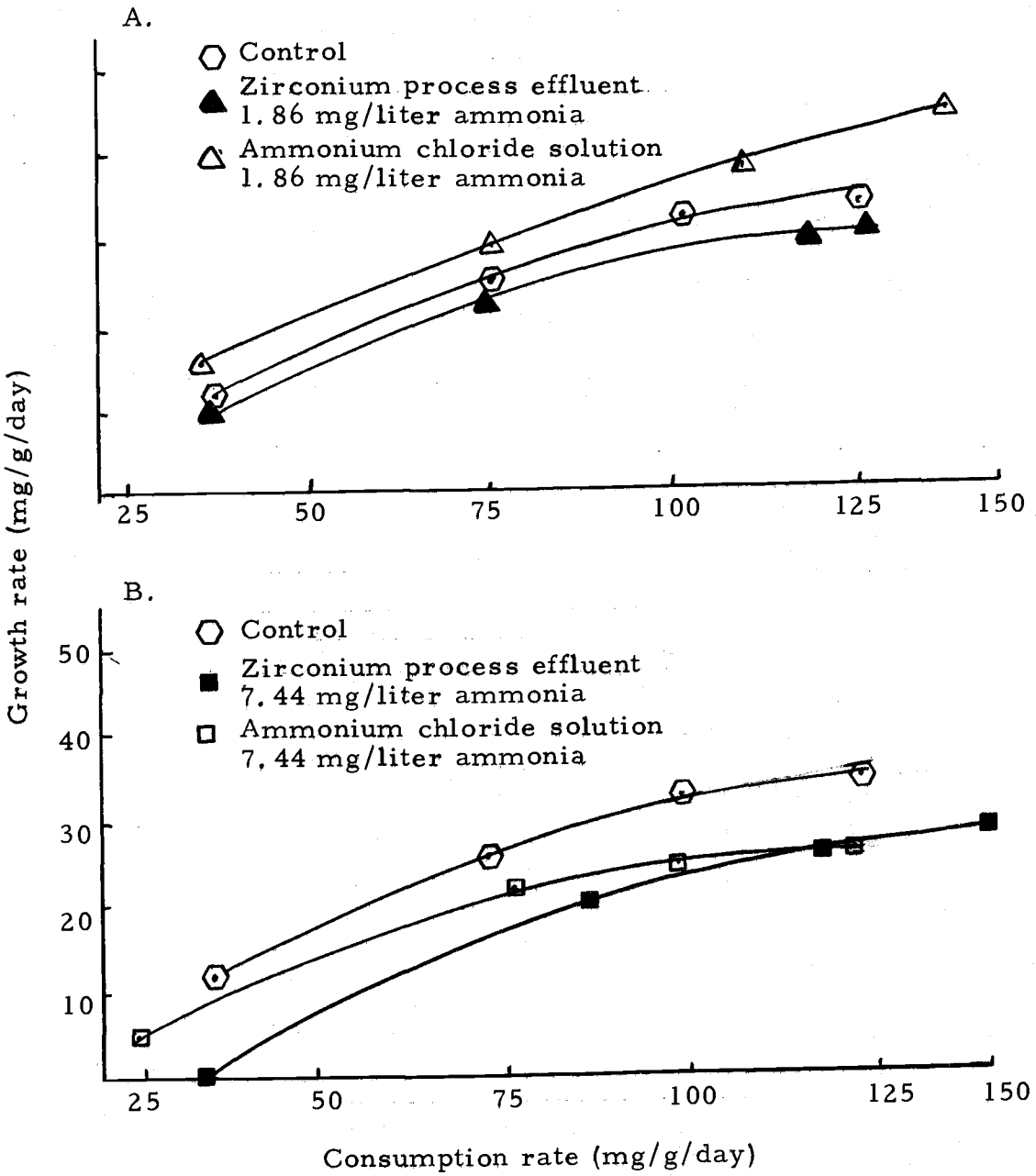

Figure 6. Relationship between growth rate and consumption rate of juvenile chinook salmon exposed to two concentrations of ammonia in either ammonium chloride or ZPE solutions. Experiment conducted at pH 7.5 and 15 C for a period of 12 days.

consumption rates studied (Figure 6b).

Fish exposed to ZPE solutions grew more slowly than their counterparts exposed to ammonium chloride solutions at all but the highest consumption rates. At the highest consumption rates the growth rate of the fish exposed to 7.44 mg/liter ammonia in ZPE solutions approaches that of the fish exposed to ammonium chloride solutions. This leads one to suspect that at this high ammonia concentration, the fish are affected so severely by the ammonia that the other toxicants in the effluent have little influence on the growth rate.

To show the relationship between growth and ammonia concentration under all conditions studied, the growth rates were normalized. Normalized growth rates were derived by dividing the growth rates of the experimental fish by the growth rates of their respective controls and multiplying by 100, thus equating the mean growth rate of each control group to 100. As the ammonia concentration increased from 0.3 mg/liter in both ammonium chloride and ZPE solutions, the normalized growth rates decreased (Figure 7). The difference between the normalized growth rates of fish exposed to ammonium chloride and ZPE solutions was greatest at 125 mg/g/day consumption rate (Figure 7a). At a consumption rate of 100 mg/g/day the difference was much smaller (Figure 7b); while at 75 mg/g/day there appeared to be no consistent difference in the normalized growth rates of fish exposed to ZPE and ammonium chloride solutions (Figure 7c). Other

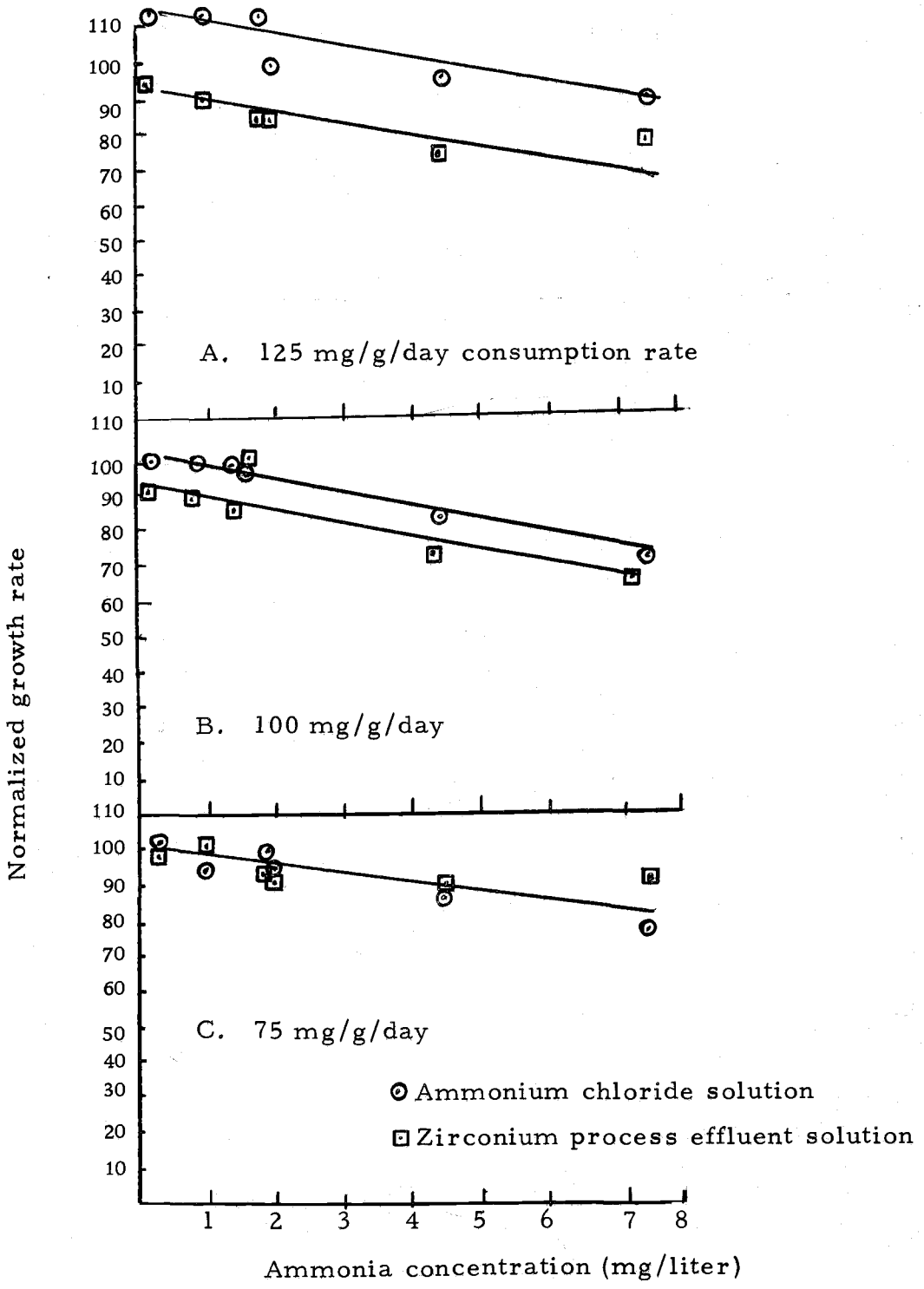

Figure 7. The normalized growth rates of juvenile chinook salmon exposed to both ammonium chloride and zirconium process effluent solutions at three different consumption rates. Experiments conducted at pH 7.5 and 15 C for 12-14 days.

toxic substances present in the ZPE appear to have the greatest effect on growth at high consumption rates.

DISCUSSION

The zirconium process effluent studied was a complex mixture of chemicals, with ammonia as the major apparent toxic constituent. The ZPE was more toxic than were ammonium chloride solutions having the same ammonia concentration, even though ammonia was the major toxic component of the effluent. Sublethal concentrations of ZPE decreased the growth rate of juvenile chinook salmon.

The ZPE was acutely toxic in each sample taken from January 1972 to January 1973. Extension of the regression line (Figure 1) to the point where a 100 percent concentration of effluent should be required to reach the 96 hr TL_{50} indicates that the ammonia concentration would be near 45 mg/liter at pH 7.5, so long as other toxic substances do not assume greater importance. In the comparative bioassays (Figure 2) the TL_{50} ammonia concentration at pH 7.5 in ZPE solutions was near 45 mg/liter. The agreement between the two values, and the fit of the regression line to the points, indicates that the regression model is satisfactory for predicting the acute toxicity of ZPE solutions, when the ammonia concentration is between 45 and 600 mg/liter. In experiments B-10, -11 (Table 2), in which the ammonia concentration was 23 mg/liter, the model does not predict the TL_{50}. If it is assumed that this ammonia concentration is on the regression line the predicted TL_{50} of the effluent would be

greater than 100 percent by volume. But since the regression line does not accurately predict the TL_{50} at this ammonia concentration, it is assumed that the toxicity is due to the other toxicants in the effluent. The acute toxicity of the other toxicants was not expressed until the ammonia reached a concentration at which it was not acutely toxic. Bliss (1939) found that when two toxicants were present in a mixture, one being more toxic than the other, the more toxic constituent may predominate until a ratio of concentrations of the two toxicants is reached at which the more toxic constituent is no longer acutely toxic. When this concentration is reached, the acute toxicity of other toxicants may be expressed. It is probable that a different regression line is needed to represent the acute toxicity of the effluent when the ammonia concentration is less than 45 mg/liter. The second regression line should reflect the acute toxicity of the other toxicants. The other toxicants present in the ZPE apparently do have some effect on the acute toxicity of the ammonia in the effluent. Finney (1947) found that other toxicants may produce nearly the same proportion of the TL_{50} over a wide range of concentrations of the secondary toxicant. This appears to be the case with ZPE, as seen in the comparative bioassays (Figure 4). As the pH is increased, the concentration of ammonia needed to reach the 96 hr. TL_{50} decreases. If the relative proportions of ammonia and the other toxicants in the ZPE are assumed to be constant, then the

concentration of the other toxicants will also decrease as the pH increases. Thus the curves representing the 96 hr TL_{50} of ammonium chloride and ZPE as unionized ammonia should approach each other as the pH increases. The curves, however, are essentially parallel, indicating that the effect of the secondary toxicants on the acute toxicity of the ZPE did not change over a wide range of concentrations.

The mode of toxicity of ammonia is not known. Visek (1954) suggested that acute ammonia toxicity in man and other animals is neurological in origin. Lloyd and Orr (1969), however, suggest that ammonia may cause death in fish by influencing osmoregulation, so that the fish accumulate an excess of water. Herbert and Shurben (1965) found the toxicity of ammonia solutions decreased as saltwater concentrations approached isotonicity. This evidence together with that found by Lloyd and Orr suggests that the mode of acute toxicity of ammonia involves the water balance of fish. Lloyd and Orr also found that concentrations of ammonia less than 2.9 mg/liter had no effect on the rate of urea production of rainbow trout. In conjunction with this, Fromm and Gillette (1968) found that a rainbow trout had abnormally high concentrations of ammonia in their blood when ambient ammonia concentrations were greater than 3.0 mg/liter. They suggested that the fish could handle the excess ammonia through excretion of other nitrogen compounds at ambient ammonia concentrations less than 3 mg/liter. Olson and Fromm (1971) found that

rainbow trout increased their rate of urea nitrogen production as the ambient ammonia concentration approached 2.5 mg/liter. At concentrations higher than 2.5 mg/liter the rate of urea nitrogen production was constant, but the total nitrogen excretion rate decreased. They suggest that rainbow trout may be able to handle ambient ammonia concentrations up to 2.5 mg/liter by the production of urea. At higher ambient ammonia concentrations the fish cannot produce urea at a high enough rate to get rid of the excess ammonia in its body. At higher ammonia concentrations, then, fish are found to have abnormally high ammonia concentrations in their blood. In the present study, juvenile chinook salmon were found to grow more rapidly than control fish at concentrations of ammonia less than 3 mg/liter. At higher ambient ammonia concentrations the growth rate of the fish was slower than that of controls.

It appears that the other toxicants present in the zirconium process effluent, either by themselves or in conjunction with the ammonia present, cause a decrease in the growth rate of juvenile chinook salmon regardless of the ammonia concentration. Juvenile chinook salmon exposed to ammonium chloride solutions having a concentration of ammonia less than 3 mg/liter grew at rates slightly higher than control fish. As the concentration of ammonia and the other toxicants in the effluent increases, the difference between the growth rates of the fish exposed to ammonium chloride solutions and

ZPE solutions remain nearly constant (Figure 8), suggesting that the effect of the other toxicants is constant over a wide range of concentrations.

Neither the acute toxicity bioassays nor the growth experiments conducted during this study monitor the conditions found below the ZPE outfall in the Willamette River. The concentrations of effluent in the river, after complete mixing, are from all indications far too low to be acutely toxic to salmonid fishes. The acute toxicity bioassays are, however, a means of identifying the toxic constituents of a complex industrial waste and monitoring its toxicity over a period of time. The growth experiments are perhaps a better indication of the toxic effects of the effluent at concentrations near those in the receiving waters. The results of the growth studies indicate that the ZPE could have sublethal effects on juvenile salmonids in the river below the outfall.

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